

Morphotaxonomic studies on *Simulium damnosum* Theobald complex (Diptera: Simuliidae) along Osun River, Southwestern Nigeria

Monsuru Adebayo ADELEKE^{1,2,*}, Chiedu Felix MAFIANA^{1,3}, Sammy Olufemi SAM-WOBO², Ganiyu Olatunji OLATUNDE⁴, Olaoluwa Pheabian AKINWALE¹

(1. Molecular Parasitology Laboratory, Public Health Division, Nigerian Institute of Medical Research, P. M. B 2013, Yaba, Lagos, Nigeria; 2. Department of Biological Sciences, University of Agriculture, Abeokuta, Nigeria;

3. Executive Secretary Office, National University Commission, Abuja, Nigeria;

4. Department of Biological Sciences, Osun State University, Osogbo, Nigeria)

Abstract: *Simulium damnosum* sensu lato is a complex made up of many sibling species which differ in their ecology and contribution to onchocerciasis transmission. The present study was carried out to provide information on morphological composition of the biting adults of *S. damnosum* s.l. along Osun River in a forest zone of Southwestern Nigeria. Adult flies were collected on human baits from 07:00 a.m. to 06:00 p.m. every fortnight at three communities, Osun Eleja, Osun Ogbere and Osun Budepo along Osun River from February 2008 to June 2009. The wing tufts and other taxonomic characters of the flies were observed and classified using standard protocol. The results revealed the sympatric existence of both forest and savanna dwelling flies. The forest flies constituted the predominant species representing 99.18% of the flies caught in the three locations while savanna dwelling flies recorded 0.82% of the total catch. The difference in abundance of the forest and savanna flies was statistically significant ($P < 0.05$). All the savanna flies encountered had pale wing tufts but there was significant difference in wing tufts colours observed among the forest flies ($P < 0.05$). Further studies are therefore recommended so as to shed light on the species composition of *S. damnosum* s.l. in the study area.

Key words: *Simulium damnosum* s.l.; morphotaxonomy; wing tufts; dominant species; Nigeria

1 INTRODUCTION

The results of various taxonomic studies had shown that there were at least nine sibling species of *Simulium damnosum* complex in western Africa and these siblings occupied different ecozones and varied in their contribution to onchocerciasis transmission (Vajime and Gregory, 1990; Boakye, 1993; Wilson *et al.*, 1993; Toe *et al.*, 1997; Dumas *et al.*, 1998). These species included *S. sirbanum*, *S. damnosum* sensu stricto, *S. dieguerense*, *S. sanctipauli*, *S. soubrense*, *S. squamosum*, *S. yahense*, *S. leonense* and *S. konkorensis* (WHO, 1994). The first three species are known as savanna flies which transmit savanna strain of *Onchocerca volvulus* while the rest belong to the forest group and transmit the forest strain of the disease of which the pathogenicity is more of skin disease with less blinding. All these siblings have also been found in other parts of Nigeria except *S. leonense*, *S. konkorensis*, *S. sanctipauli* and *S. dieguerense* (Mafuyai *et al.*, 1996; Ibeh *et al.*, 2006).

However, the identification of sibling species of *S. damnosum* complex has been mainly on cytological approach, *i. e.* based upon the analysis of the polytene chromosomes from the larval silk gland. The cytotaxonomic method is also specific in that it can only be carried out on 7th instar larvae of the flies, therefore making it impossible to identify the adult flies which are actual stage transmitting *O. volvulus* (Ibeh *et al.*, 2008). In view of the rapid change in population structure of the biting adult flies in many localities coupled with the contribution of each sibling species to the epidemiology of onchocerciasis, the accurate identification of the adult flies becomes imperative for proper understanding of the disease epidemiology and for the rational design of disease control measures (Mustapha *et al.*, 2004). Such information is useful in monitoring the changes in epidemiological pattern of disease transmission which may be brought about by changes in species composition.

Morphotaxonomy has remained the rapid method of adult identification in the field (Mafuyai *et al.*,

* Corresponding author, E-mail: healthbayom@yahoo.com

Received: 2010-03-24; Accepted: 2010-10-13

1997; Ibeh *et al.*, 2008). Wilson *et al.* (1993) reviewed and developed the methods for morphological discrimination of adult females based on the earlier works of Garms *et al.* (1982) and Garms and Zillman (1984). The use of morphological characters could allow the identification of *S. yahense* with approximately 99% specificity while the savanna cytospecies could be easily separated from the forest dwelling species using the colour of the wing tufts, colouration of fore coxa and abdominal tergite (Kurtak *et al.*, 1981; Garms *et al.*, 1982; Wilson *et al.*, 1993; Mafuyai *et al.*, 1996; Mank *et al.*, 2004). Though there are reported studies on species composition on biting adults in some river systems in Nigeria (Mafuyai *et al.*, 1997; Ibeh *et al.*, 2008; Oluwole *et al.*, 2009), there is no reported study on fly composition of the biting adults of *S. damnosum* s.l. along Osun River, Southwestern Nigeria. Moreover, there is paucity of information on morphological composition of *S. damnosum* s.l. in Southwestern Nigeria. It is against this background that the present study was carried out to investigate the morphological composition of the biting adults of *Simulium damnosum* s.l. along Osun River, Southwestern Nigeria.

2 MATERIALS AND METHODS

2.1 The study area

The study was conducted along Osun River system, Southwestern Nigeria. Osun River lies on the latitude 8°20' and 6°30' N and longitude 5°10' and 3°25' E in the forest zone of Nigeria. Both forest transitional zone and rain forest are found along the river course. Generally, Southwestern Nigeria usually has the annual rainfall ranging from 1 000 mm to 1 600 mm and it experiences two seasons, the wet season (April to October) and the dry season (November to March). Three catching points, Osun Eleja (derived savanna), Osun Budepo (rainforest) and Osun Ogbere (rainforest), were selected along the river course representing the two ecological zones along Osun River system. Osun Eleja is located on latitude 7°16' N and longitude 4°08' E while Osun Budepo is located on latitude 7°04' N and longitude 4°08' E. Osun Ogbere is located on the latitude 6°76' N and longitude 4°13' E (Fig. 1).

2.2 Collection of adult flies

Adult flies were collected fortnightly on human bait in the three catching points in accordance with the standard protocol between February 2008 to January 2009 in Osun Budepo and Osun Ogbere and

July 2008 to June 2009 in Osun Eleja. Two adult fly collectors were positioned near the bank of the river in each of the catching sites. The two fly capturers catch flies alternately between 07:00 a. m. to 06:00 p. m. by exposing the lower portion of their legs. Any fly coming to bite the exposed legs was caught using the catching tube. The flies caught were pooled according to the hour of catch in each location.

2.3 Morphotaxonomy of the adult flies

All the flies caught were identified using morphotaxonomic characters with the aid of dissecting microscope. The colours of the forecoxa, scutellar hairs and ninth abdominal tergite of each of the fly were observed and scored as pale, dark or intermediate as described by Wilson *et al.* (1993) to classify the fly into forest or savanna dwelling group. The wing tufts (stem vein setae) were also observed and scored on a scale A – E (otherwise known as 01 – 05) and 0 in accordance with Kurtak *et al.* (1981).

2.4 Data analysis

The data obtained were subjected to *t*-test analysis to determine the significant differences in the parameters determined.

3 RESULTS

3.1 Relative abundance of the forest and savanna dwelling flies

The results of morphotaxonomy revealed the presence of both forest and savanna dwelling flies. The relative abundance of the forest and savanna flies at the study locations during normalized capture is presented in Table 1. Forest dwelling flies were the predominant flies representing 99.18% of the flies caught in the three locations while savanna dwelling flies were 0.82% of the total. Forest flies were also the dominant flies in each of the locations. The forest flies were 99.13%, 98.11% and 99.52% of the total of the flies caught at Osun Budepo, Osun Ogbere and Osun Eleja, respectively (Table 1). A significant difference was observed between the abundance of the forest dwelling flies and that of savanna dwelling flies in the three locations ($t = -2.91$, $P < 0.05$).

3.2 Seasonal occurrence of the savanna and forest dwelling flies

The monthly data on morphotaxonomy of the flies (Fig. 2) revealed that savanna flies occurred in three out of twelve months at Osun Budepo. The occurrence of savanna flies was recorded in February, March and April while the forest flies allopatrically occurred in other months of the year.

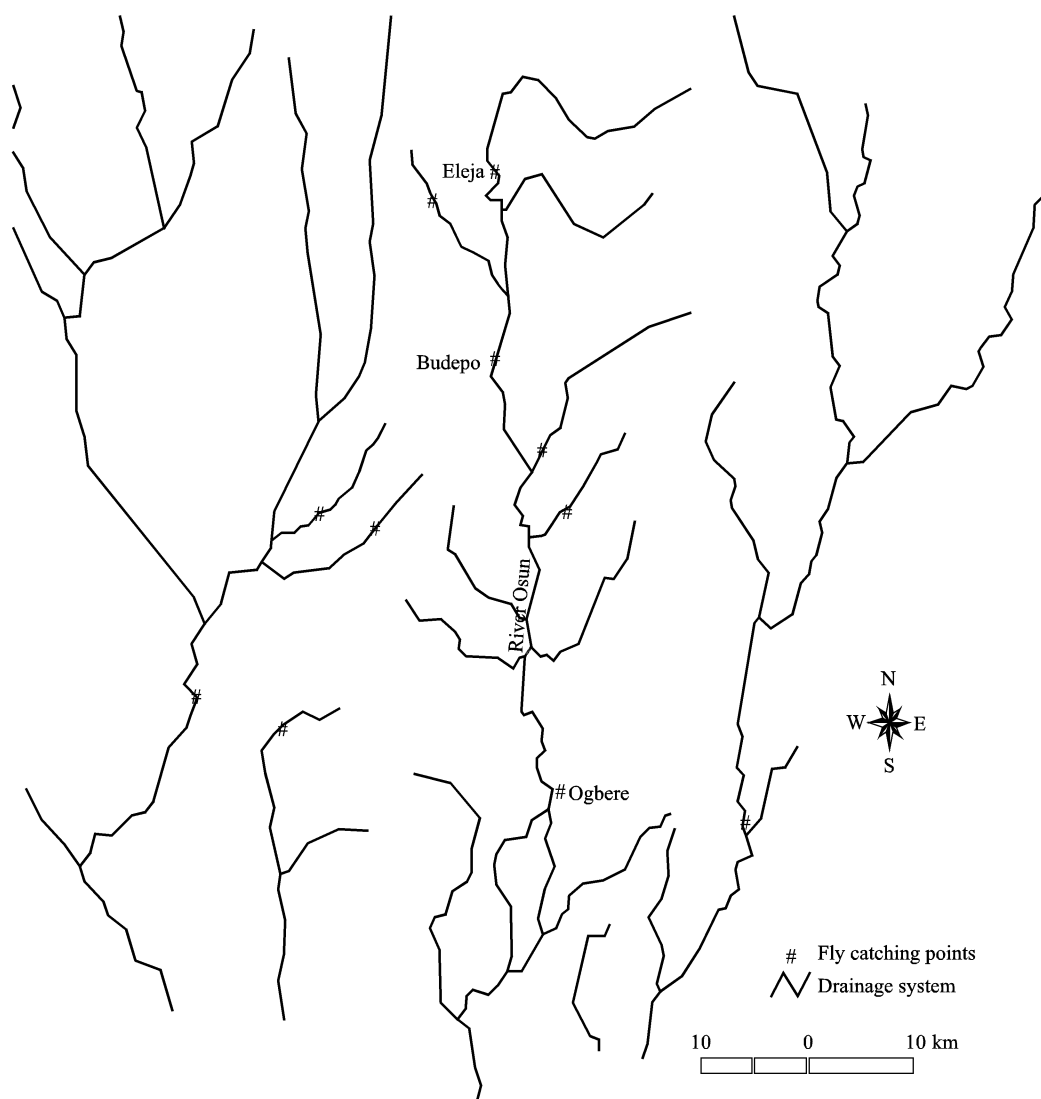


Fig. 1 Map of Osun River system showing the catching points

At Osun Ogbere, the savanna flies were caught only in February, March and April. The forest flies predominate in the remaining months except December and January when no fly was caught at the site. At Osun Eleja, the savanna flies were only caught in February and March while the forest flies were encountered in all the months of the study except February when equal number of savanna and forest flies occurred.

Table 1 The relative abundance of savanna and forest flies at the three catching points during the study period

Types of flies	Osun Budepo	Osun Ogbere	Osun Eleja	Total
Savanna group	6(0.87)	3(1.89)	3(0.48)	12(0.82)
Forest group	686(99.13)	156(98.11)	618(99.52)	1447(99.18)
Total	692	159	621	1472

Data are the number of flies, while those in parentheses are the percentages of the total.

3.3 Morphological variation of the flies

All the savanna flies encountered have completely pale wing tufts (A). However, there was variation in the wing tufts colours found among the forest flies in the three sites. Flies with the wing tufts E (05) had the highest number of occurrence in the three sites followed by wing tufts D (04). The flies with wing tufts C (03) recorded the least occurrence at Osun Eleja and Osun Ogbere as against A (01) which recorded the least at Osun Budepo (Fig. 3). All the flies without visible wing tufts (0) had dark forecoxa and were classified as forest flies. All the forest flies had dark forecoxa and antennae but pale scutellum and pale abdominal tergite. The variation in the occurrence of wing tufts colours among the forest flies was observed to be statistically significant in each of the study sites ($P < 0.05$).

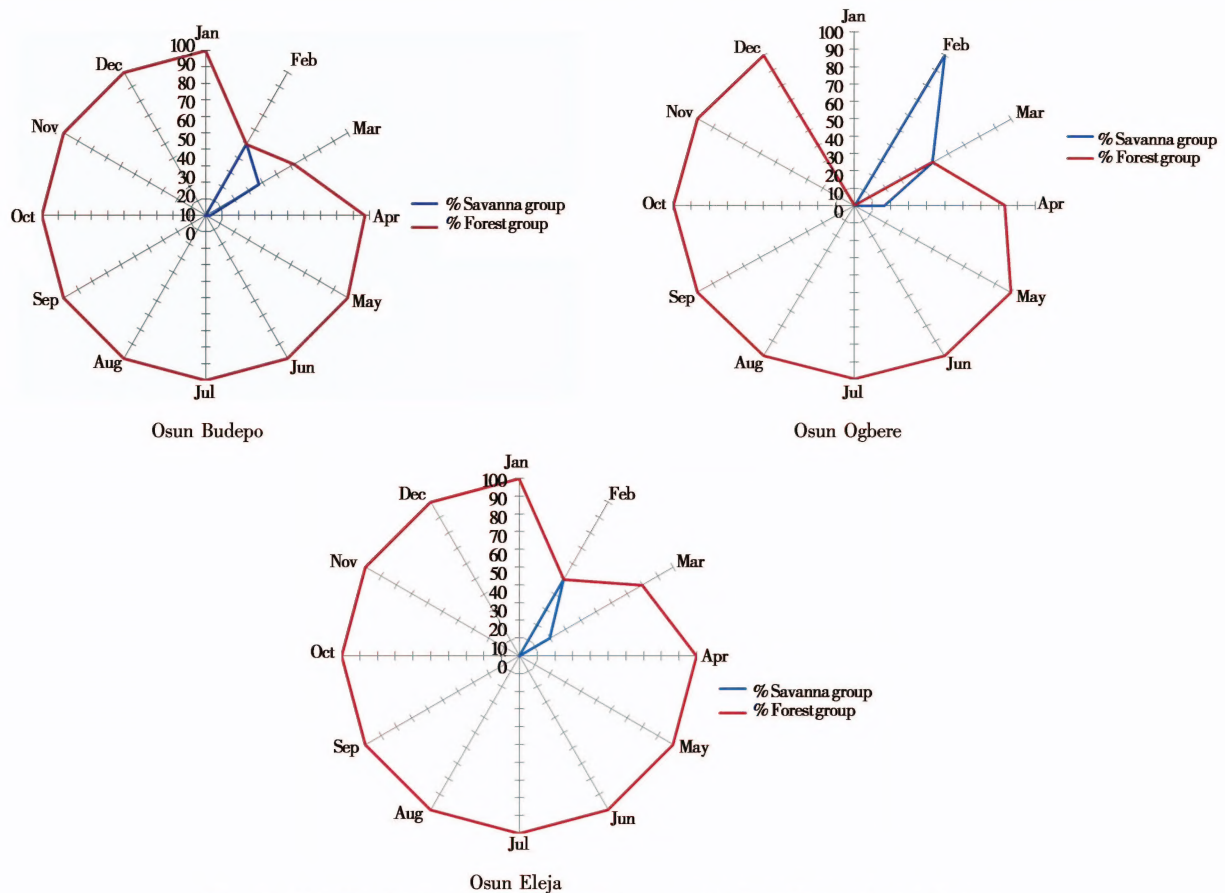


Fig. 2 Monthly distribution of forest and savanna dwelling flies at the study sites

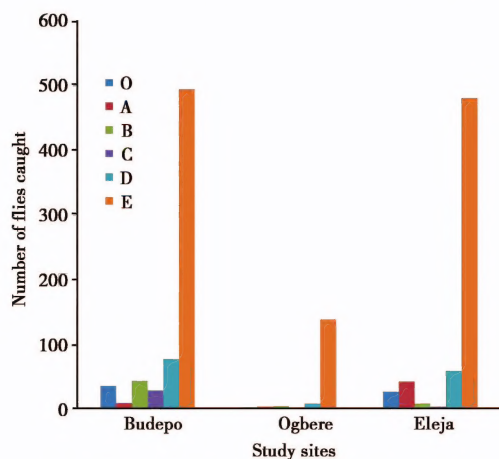


Fig. 3 Variation in wing tufts colours among forest flies at the catching sites

O; No visible wing tufts; A; All the wing tufts pale; B; More pale wing tufts with few dark ones; C; Mixed wing tufts; D; More dark wing tufts with few pale ones; E; All the wing tufts dark.

4 DISCUSSION

The results of the morphotaxonomic studies revealed the sympatric composition of both forest and savanna dwelling flies along Osun River. The preponderance of the forest flies is expected since Osun River is located in the forest zone of Nigeria. This

observation probably confirms the earlier reports that ecology plays a key role in the distribution of the siblings of *S. damnosum* s. l. (Grunewald, 1976; WHO, 1994). However, the presence of the savanna flies is worrisome and this could have been caused by deforestation, climatic changes or wind borne migration. Though, the number of savanna flies caught at the three catching points is very low, the invasion of the savanna flies into southern part where only the forest flies are expected to exist is dangerous because the highly pathogenic form of onchocerciasis could soon be found in the area where only the benign forest form is thought to exist, as savanna flies could carry along the blinding form of *O. volvulus* during migration (Opoku, 2006).

The variation observed in the wing tufts of the forest flies at the three catching points confirms the limitation of wing tufts as a sole diagnostic character. Using the wing tufts alone, the appreciable number of the forest flies would have been misidentified as savanna flies based on pale wing tufts. Though, there was significant variation in wing tufts colours of the forest flies at the three sites, whether these flies actually differed in species composition or the variation is an outward phenotypic expression of offspring of different variation are limitations that cannot be

established by morphotaxonomy. In western Africa, *S. damnosum* s. l. has been known to consist of nine sibling species (Toe *et al.*, 1997). Among the nine siblings, the distribution of *S. leonense*, *S. konkorensis*, *S. dieguerensis* and *S. sanctipauli* is restricted to only Sierra Leone, Guinea and Mali, respectively (Garms, 1973; Grunewald, 1976; Fryauff and Trpis, 1986). The absence of completely dark flies in the present study also eliminates the possibility of the existence of *S. yahense* in the study area. *S. yahense* has a characteristic dark wing tufts, fore coxa, scutellum, antennae and ninth abdominal tergite, and these characters are unmistakably used to distinguish this sibling from others (Wilson *et al.*, 1993). The preference of *S. yahense* for a small well shaded river (Fryauff and Trpis, 1986; Mafiana, 1990) could have accounted for its absence since the reverse of this requirement is found along Osun River.

Therefore, based on the present study, an inference could be drawn that the sibling species likely to be present along Osun River belong to *S. damnosum*, *S. sirbanum*, *S. squamosum* or *S. soubrensis*. Further studies are recommended on the molecular characterization of the flies so as to shed light on their species status.

ACKNOWLEDGEMENTS The authors thank the residents of the study communities and the fly collectors for their assistance during this study.

References

- Boakye DA, 1993. A pictorial guide to the chromosomal identification of members of the *Simulium damnosum* Theobald complex in West Africa with particular reference to the Onchocerciasis Control Programme Area. *Annals of Tropical Medicine and Parasitology*, 44: 223 – 244.
- Dumas V, Herder S, Bebb A, Cadoux-Barnabe C, Bellec C, Cuny G, 1998. Polymorphic microsatellites in *Simulium damnosum* s. l. and their use for differentiating two savannah populations: implications for epidemiological studies. *Genome*, 41: 154 – 161.
- Fryauff DJ, Trpis M, 1986. Identification of larval and adult *Simulium yahense* and *Simulium sanctipauli* based on species-specific enzyme markers and their distribution at different breeding habitats in Central Liberia. *Am. J. Trop. Med. Hyg.*, 35(6): 1218 – 1230.
- Garms R, 1973. Quatitative studies on the transmission of *Onchocerca volvulus* by *Simulium damnosum* in the Bong Range, Liberia. *Tropenmed. Parasit.*, 24: 358 – 372.
- Garms R, Cheke RA, Vajime CG, Sowah S, 1982. The occurrence and movements of different members of the *Simulium damnosum* complex in Togo and Benin. *Z. Ang. Zool.*, 69: 219 – 236.
- Garms R, Zillman U, 1984. Morphological identification of *Simulium sanctipauli* and *S. yahense* in Liberia and comparison of results with those of enzyme electrophoresis. *Tropenmedizin und Parasitologie*, 35: 217 – 220.
- Grunewald J, 1976. The hydro-chemical and physical conditions of the environment of the immature stages of some species of the *Simulium* (*Edwardsellum*) *damnosum* complex (Diptera: Simuliidae). *Tropenmed. Parasit.*, 27: 438 – 454.
- Ibeh OO, Nwoke BEB, Adegoke JA, 2008. Morphological differentiation of vectors of onchocerciasis, *Simulium damnosum* complex, in south-east Nigeria. *Nigerian Journal of Parasitology*, 29(1): 61 – 66.
- Ibeh OO, Nwoke BEB, Adegoke JA, Mafuyai HB, 2006. Cytospecies identifications of vectors of human onchocerciasis in south eastern Nigeria. *African Journal of Biotechnology*, 5(19): 1813 – 1818.
- Kurtak DC, Raybould JN, Vajime CG, 1981. Wing tuft colours in the progeny of single individuals of *Simulium squamosum* (Enderlein). *Transaction of the Royal Society of Tropical Medicine and Hygiene*, 75: 126.
- Mafiana CF, 1990. Morphometric studies of female *Simulium damnosum* s. l. from a rainforest village in Bendel State of Nigeria. *Bioscience Research Communication*, 2(2): 191 – 195.
- Mafuyai HB, Post RJ, Molyneux DH, Davies DH, 1997. First sibling species identification of Nigerian onchocerciasis vectors. *Transaction of the Royal Society of Tropical Medicine and Hygiene*, 91: 90 – 91.
- Mafuyai HB, Post RJ, Vajime CG, Molyneux DH, 1996. Cytotaxonomic identifications of the *Simulium damnosum* complex (Diptera: Simuliidae) from Nigeria. *Tropical Medicine and International Health*, 1: 779 – 785.
- Mank R, Wilson MD, Rubio JM, Post RJ, 2004. A molecular marker for the identification of *Simulium squamosum* (Diptera: Simuliidae). *Annals of Tropical Medicine and Parasitology*, 98(2): 197 – 208.
- Mustapha M, Post RJ, Enyong P, Lines J, 2004. A new cytotype of *Simulium squamosum* from south-west Cameroon. *Medical and Veterinary Entomology*, 18: 296 – 300.
- Oluwole AS, Ekpo UF, Mafiana CF, Adeofun CO, Idowu OA, 2009. Preliminary study on temporal variations in biting activity of *Simulium damnosum* s. l. in Abeokuta North LGA, Ogun State Nigeria. *Parasite and Vector*, 2(55): 1 – 3.
- Opoku AA, 2006. The ecology and biting activity of black flies (Simuliidae) and the prevalence of onchocerciasis in an Agricultural Community in Ghana. *West Africa Journal of Applied Ecology*, 9: 1 – 7.
- Toe L, Tang J, Back C, Katholi CR, Unnasch TR, 1997. Vector-parasite transmission complexes for onchocerciasis in West Africa. *Lancet*, 349: 163 – 166.
- Vajime CG, Gregory WG, 1990. Onchocerciasis species complex of vectors and epidemiology. *Acta Leidensia*, 59: 235 – 252.
- WHO, 1994. 25 Years of Onchocerciasis Control Programme in West Africa, WHO Report, Geneva. 235 pp.
- Wilson MD, Post RJ, Gomulski LM, 1993. Multivariate morphotaxonomy in the identification of adult female *Simulium damnosum* Theobald complex (Diptera: Simuliidae) in the Onchocerciasis Control Programme area of West Africa. *Annals of Tropical Medicine and Parasitology*, 87: 65 – 82.

尼日利亚西南部奥孙河沿线黑蝇 *Simulium damnosum* Theobald complex 的形态分类研究 (双翅目: 蚋科)

Monsuru Adebayo ADELEKE^{1,2,*}, Chiedu Felix MAFIANA^{1,3},
Sammy Olufemi SAM-WOBO², Ganiyu Olatunji OLATUNDE⁴,
Olaoluwa Pheabian AKINWALE¹

(1. Molecular Parasitology Laboratory, Public Health Division, Nigerian Institute of Medical Research, P. M. B 2013, Yaba, Lagos, Nigeria;

2. Department of Biological Sciences, University of Agriculture, Abeokuta, Nigeria; 3. Executive Secretary Office,
National University Commission, Abuja, Nigeria; 4. Department of Biological Sciences, Osun State University, Osogbo, Nigeria)

摘要: 黑蝇 *Simulium damnosum* sensu lato 是由多个姊妹种组成的复合体,这些种在生态学和盘尾丝虫病的传播方面各不相同。本文对奥孙河沿线的尼日利亚西南部森林区域的黑蝇 *S. damnosum* s.l. 复合体的组成以及成虫的形态学特征进行了研究。本研究所用的黑蝇 *S. damnosum* s.l. 成虫标本来源于奥孙河沿线的 3 个区,分别是 Osun Eleja, Osun Ogbere 和 Osun Budepo。标本采集通过人体诱捕的方式,采集时间从 2008 年 2 月至 2009 年 6 月上午 7:00 到下午 6:00,每两周采集 1 次。通过观察成虫的翅毛簇(wing tufts)和其他形态特征,对其进行分类研究。结果表明:存在同域分布的森林种和稀树草原种。在奥孙河沿线 3 个区内,森林种为优势种,占总捕获量的 99.18%,而稀树草原种仅占 0.82%;森林种和稀树草原种在多样性上存在显著差异($P < 0.05$)。所捕获的所有稀树草原种的翅毛簇均为灰白色,而捕获的森林种的翅毛簇颜色存在显著差异($P < 0.05$)。为了更清楚地揭示该地区黑蝇 *S. damnosum* s.l. 的物种组成,建议进行更深入的研究。

关键词: *Simulium damnosum* s.l.; 形态分类学; 翅毛簇; 优势种; 尼日利亚

中图分类号: Q969 **文献标识码:** A **文章编号:** 0454-6296(2010)11-1319-06

(责任编辑: 袁德成)